# Living Colors™ Fluorescent Proteins


**A revolution in color.**

CLONTECH
NOW YOU CAN.

# Living Colors™ Fluorescent Proteins

*Revolutionary reporters for monitoring gene expression and protein localization*

*The cloning of green fluorescent protein from the jellyfish* Aequorea victoria *(1) has revolutionized the way we use reporter molecules to study biological phenomena. This remarkable molecule, GFP, is the cornerstone of CLONTECH's* ***Living Colors™ Fluorescent Protein Products****—a complete line of vectors and reagents for all of your expression needs. With these products, you can monitor gene expression* in vivo, in situ, *and in real time.*

## Choose from a spectrum of enhanced color variants

CLONTECH continues to bring you the latest advances in fluorescent protein technology. We now offer enhanced GFP variants in four colors—green, blue, yellow, and cyan (Table I). These color variants are all available in human codon-optimized formats for optimum expression in higher eukaryotes. Because these variants all have distinct spectra, you can perform double- and triple-labeling experiments.

## Optimize your studies with EGFP

Enhanced GFP (EGFP; 2, 3) is a carefully optimized variant that is available exclusively from CLONTECH. Whereas the limit of detection of wild-type GFP is ~1 µM, EGFP can be detected at expression levels as low as ~100 nM. This is equivalent to roughly 10,000 molecules in a cell's cytoplasm, or only 2,000 molecules on its surface (4). Modified forms, such as destabilized EGFP and farnesylated EGFP, are available for specialized applications.

## Monitor fluorescence in living cells or organisms

Unlike other bioluminescent reporter molecules, GFP itself emits bright green light when excited by UV or blue light. No additional cofactors or substrates are required, so processes occurring in living cells and whole organisms can be monitored as they occur without fixation or disruption.

## Analyze expression in living or fixed samples

GFP fluorescence is stable over several days to weeks in living cells. In fixed cells, fluorescence can be detected even after more than three months. Moreover, fusions of GFP to other proteins retain fluorescence.

## Proven performance in a wide range of hosts

Our Living Colors products have been referenced more than 100 times in scientific journal articles. GFP has been shown to fluoresce in a wide variety of organisms, including bacteria, yeast, Drosophila, *C. elegans*, zebrafish, Xenopus, mice, human cells, and a variety of plants.

## Valuable tools for a variety of applications

The photographs on these pages show just a few of the myriad applications of CLONTECH's Living Colors Fluorescent Proteins. To learn more about what you can do with these products, just turn the page...

## Transgenics

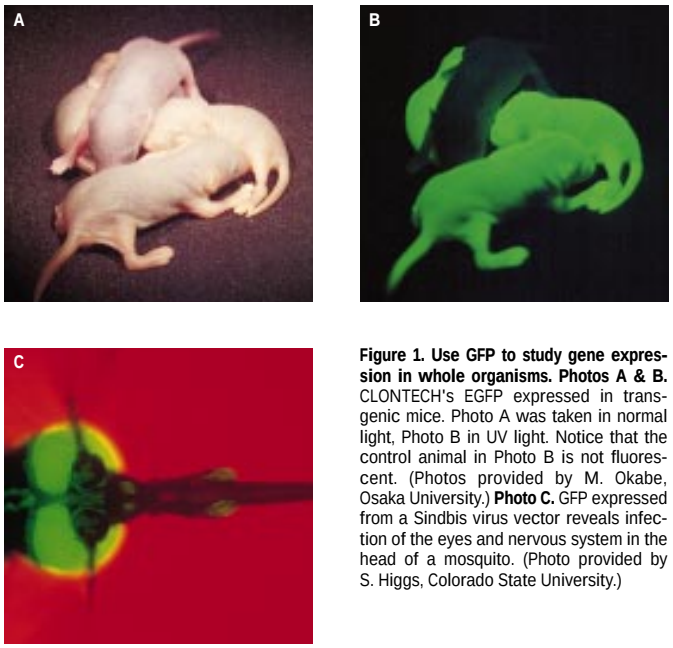


**Figure 1. Use GFP to study gene expression in whole organisms. Photos A & B.** CLONTECH's EGFP expressed in transgenic mice. Photo A was taken in normal light, Photo B in UV light. Notice that the control animal in Photo B is not fluorescent. (Photos provided by M. Okabe, Osaka University.) **Photo C.** GFP expressed from a Sindbis virus vector reveals infection of the eyes and nervous system in the head of a mosquito. (Photo provided by S. Higgs, Colorado State University.)

WINNER OF R&D AWARD

**Visit gfp.clontech.com for vector maps, citations, and a complete list of Living Colors™ products.**



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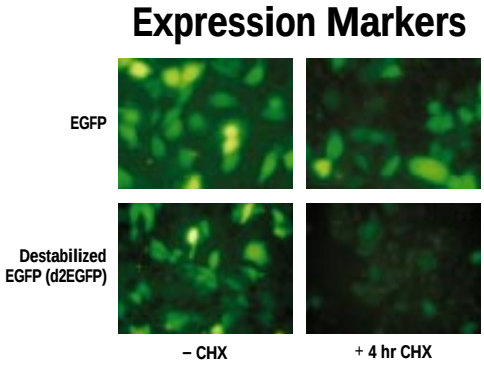


**Figure 2. Destabilized variants for rapid turnover *in vivo*.** CHO-K1 cells constitutively expressing either d2EGFP or EGFP were treated with cycloheximide (CHX) to inhibit protein synthesis. After 4 hr of treatment with CHX, fluorescence intensity of cells expressing d2EGFP was greatly reduced (lower right panel).

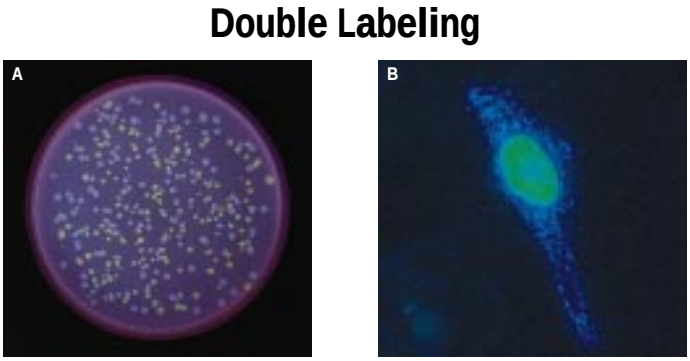


**Figure 3. EYFP and ECFP—the perfect pair for standard dual-color fluorescence microscopy. Photo A.** Yellow and cyan fluorescent JM109 colonies. Cells were transformed separately with pEYFP and pECFP and mixed before plating. **Photo B.** Localized nuclear and mitochondrial fluorescence in HeLa cells after transfection with pEYFP-Nuc and pECFP-Mito.

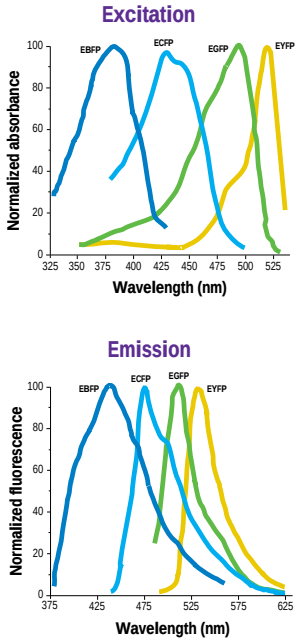


**Figure 4. Excitation and emission spectra for enhanced fluorescent proteins.** (Data provided by G. Patterson & D. Piston, Vanderbilt University.)

**Table I: Living Colors Fluorescent Proteins**

| Protein | Color | Excitation/ Emission Maxima (nm) | Features/Applications |
|---|---|---|---|
| **Standard enhanced color variants** | | | |
| EBFP | blue | 380/440 | Double and triple labeling |
| ECFP | cyan | 433 & 453/ 475 & 501 | Double and triple labeling |
| | | | Less photobleaching than EBFP |
| EGFP | green | 488/509 | Most widely used color variant |
| | | | Farnesylated variant available for use with ethanol-fixed samples |
| EYFP | yellow-green | 513/527 | Double and triple labeling |
| **Destabilized variants** | | | |
| d1EGFP, d2EGFP, d4EGFP | green | 488/509 | Quantitative reporter assays in mammalian cells |
| GFP(ASV), GFP(AAV), GFP(LVA) | green | 501/511 | Quantitative reporter assays in bacteria |

# Dynamic Localization Studies

## Visualize biological processes as they occur

Living Colors proteins can be used in living cells and organisms without disrupting essential biological processes. By expressing a fusion of your protein of interest to an enhanced fluorescent protein, you can easily track your protein's expression in real time. This is clearly demonstrated by the EGFP-expressing transgenic mice shown in Figure 1 (page 1). In transparent organisms, such as *C. elegans* and zebrafish, fluorescence can be observed in living organisms.

## Perform double-labeling experiments with ease

The different color variants can be used in combination for a variety of multicolor fluorescence applications, such as the ones shown in Figure 3 (page 2). Other promising applications include analysis by flow cytometery of mixed cell populations and monitoring gene expression from two different promoters in the same cell, tissue, or organism; ECFP and EYFP make the ideal pair for standard dual-color fluorescence microscopy. By using digital imaging microscopy, it is also possible to triple label cells with ECFP, EBFP, and EYFP (5).

## Label subcellular organelles and structural proteins

Our subcellular localization vectors allow you to visualize many of the dynamic processes that occur within cells. Each of these vectors contains an enhanced color variant fused to a sequence that targets it to a particular region of the cell (6). For instance, the fusion protein expressed by pEGFP-Tub can be used to visualize a cell's filamentous tubulin network (Figure 5C), allowing you to track dynamic microtubule assembly in living or fixed cells. Likewise, the pEYFP-Mito protein is translocated to the mitochondrion, producing bright yellow-green fluorescence throughout its lumen. These vectors will soon be available with a choice of different color variants, for performing double-labeling experiments. Other localization markers will also be available.

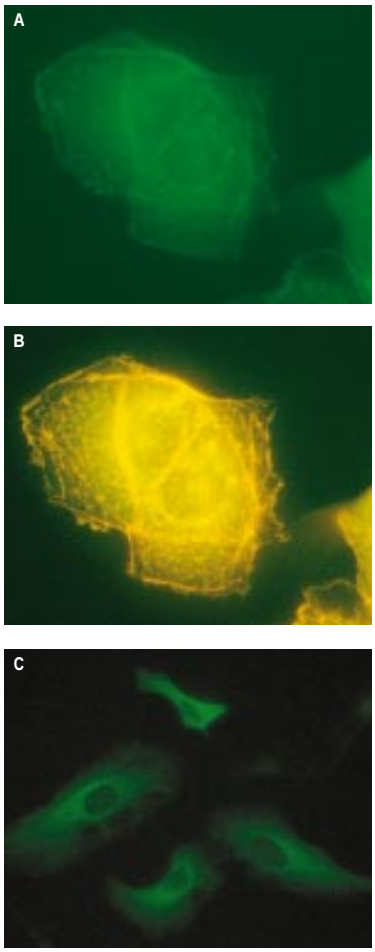


**Figure 5. EGFP fluorescence tracks microtubule assembly in real time. Photos A & B.** Stable expression of EGFP-actin in MDCK cells. Photo A shows incorporation of EGFP into actin filaments; Photo B is a double exposure showing fluorescence of EGFP-Actin and rhodamine-labeled phalloidin, a dye that specifically binds actin filaments. **Photo C.** Incorporation of EGFP into tubulin in HeLa cells using pEGFP-Tub.

### References


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# Gene Expression Analysis

## Perform quantitative and kinetic expression assays

CLONTECH's destabilized GFP variants feature rapid rates of turnover in mammalian or bacterial expression systems, and are ideal for use in quantitative reporter assays and kinetic studies (5). Because of their rapid turnover rates, destabilized variants can be used to track cellular processes as they occur. As with our other enhanced fluorescent proteins, expression of destabilized EGFP variants can be easily and immediately visualized by fluorescence microscopy (Figure 3) or analyzed by flow cytometry. For mammalian expression, destabilized enhanced GFP (dEGFP) variants are available with half-lives of one, two, and four hours (Figure 6A). These dEGFP variants were constructed by adding sequences that target proteins for rapid turnover (5). All three variants are human codon-optimized and retain the same spectral properties as the bright EGFP molecule. The Bacterial Destabilized GFP Vector Set contains three destabilized GFP variants with approximate half-lives of 40, 60, and 110 minutes (Figure 6B); actual half-lives may vary depending on the host. Each dGFP variant has a different C-terminal peptide tag that targets it for degradation by endogenous tail-specific proteases in Gram-positive or Gram-negative bacteria (7, 8). Mammalian and bacterial variants with different half-lives provide you with a choice for your particular organism, experimental design, and detection method.

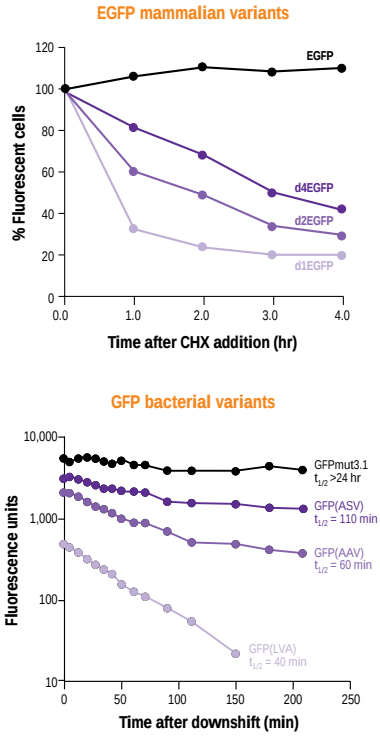


**Figure 6. CLONTECH's destabilized variants are available with several different half-lives to suit a variety of experimental designs. EGFP mammalian variants.** CHO-K1 cells transiently expressing EGFP, d1EGFP, d2EGFP, or d4EGFP were treated with CHX, an inhibitor of protein synthesis, and collected at various time points for flow cytometry analysis. **GFP bacterial variants.** *E. coli* cells expressing destabilized GFP variants were transferred to minimal medium without inducer and harvested at various time points. Fluorescence intensities were measured using a fluorometer at 515 nm. (Data for bacterial variants provided by Dr. Claus Sternberg, Technical University of Denmark.)

## Investigate promoter and enhancer elements

Promoterless enhanced fluorescent protein vectors are available for monitoring the activity of promoters or enhancer elements cloned upstream of the gene encoding the fluorescent protein. These vectors can be used to characterize *cis*-acting transcriptional control elements in living bacterial or mammalian cells. The use of EGFP variants as reporters allows for real-time measurement of transient promoter activity. These vectors can be used for flow cytometry, fluorometry, or fluorescence microscopy applications.

## Identify regulators of signal transduction pathways

CLONTECH's transcription reporter vectors are valuable tools for researchers who want to monitor the activation of specific signal transduction pathways. With these vectors, you can quickly and easily screen for potential regulators of the signal transduction pathways of interest. Identifying agonists and antagonists of various signal transduction pathways may lead to new therapies for diseases such as cancer and immunological disorders. Watch for more transcription reporter vectors in future issues of *CLONTECHniques* and at gfp.clontech.com!

# Coexpression Applications

## Save time and resources while developing functional, stable cell lines

CLONTECH's pIRES2-EGFP and pIRES-EYFP Bicistronic Vectors are designed for the simultaneous expression of EGFP or EYFP and a gene of interest from the same promoter in mammalian cells. These vectors contain the internal ribosome entry site (IRES) of the encephalomyocarditis virus. During translation, the ribosome can enter the bicistronic mRNA either at the 5' end to translate the gene of interest, or at the IRES to translate EGFP or EYFP. Thus, nearly 100% of EGFP- or EYFP-expressing cells will also express your protein of interest (9–11). Transfected cells can be easily selected by flow cytometry (Figure 7) and used directly in assays, so you can eliminate transfection efficiency as a variable in your experiments.

## Visually select for stable mammalian cell lines expressing your gene of interest

For the creation of stable mammalian cell lines, you can cotransfect one of CLONTECH's dual-selection marker vectors with a vector expressing your gene of interest (12). These bifunctional marker vectors express fusions of popular drug resistance markers with EGFP, so they provide a convenient combination of traditional drug selection and simple visual selection. These vectors are designed to be cotransfected with an expression vector containing a gene of interest at a ratio of 1:10 to 1:100 of marker to expression vector, increasing the likelihood that all cells expressing the fusion protein will also express your gene of interest. After transfection, the cell population can be enriched for positive clones by flow cytometry to detect EGFP expression.

## Monitor transfection efficiencies in living or fixed samples

Farnesylated EGFP (EGFP-F) is an excellent cotransfection marker for determining efficiency of transfection with a vector containing your gene of interest (5). The pEGFP-F Vector was created by fusing the C-terminal Ras farnesylation signal in-frame with the C-terminus of EGFP, a modification that targets EGFP-F to the plasma membrane. This feature makes EGFP-F particularly useful for transgenic studies that require tissue fixation in ethanol. EGFP-F's specific localization allows it to be easily viewed by fluorescence microscopy (Figure 8). After transfection, sorting cells for EGFP-F fluorescence by flow cytometry can decrease the need for multiple rounds of antibiotic selection.

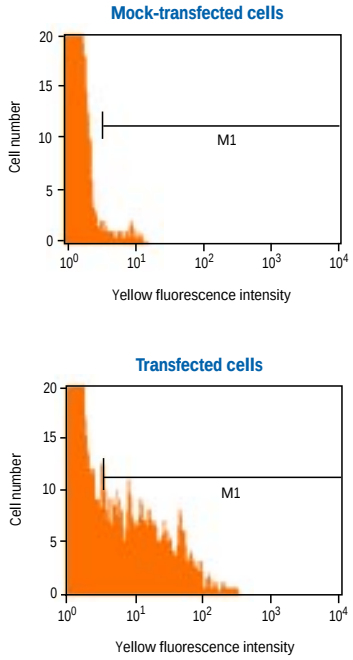


**Figure 7. Mammalian cells transfected with pIRES-EYFP can be analyzed by flow cytometry.** EL-4 (murine thymoma) cells expressing EYFP were analyzed by flow cytometry using 488-nm excitation. 20% of the cells transfected with pIRES-EYFP show significantly higher fluorescence than mock-transfected cells. (Data provided by L. Lybarger & R. Chervenak, Louisiana State University.)

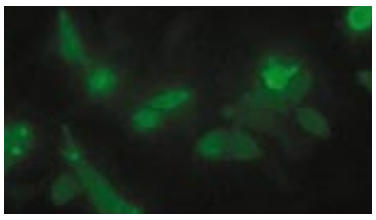

**Figure 8. Farnesylated EGFP is strongly localized to the plasma membrane for visualization in living and fixed cells.** The pEGFP-F Vector was transfected into CHO-K1 cells using CLONfectin™. After 24 hr, cells were rinsed in PBS, fixed in neutral buffered formalin for 20 min, and observed by fluorescence microscopy.

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